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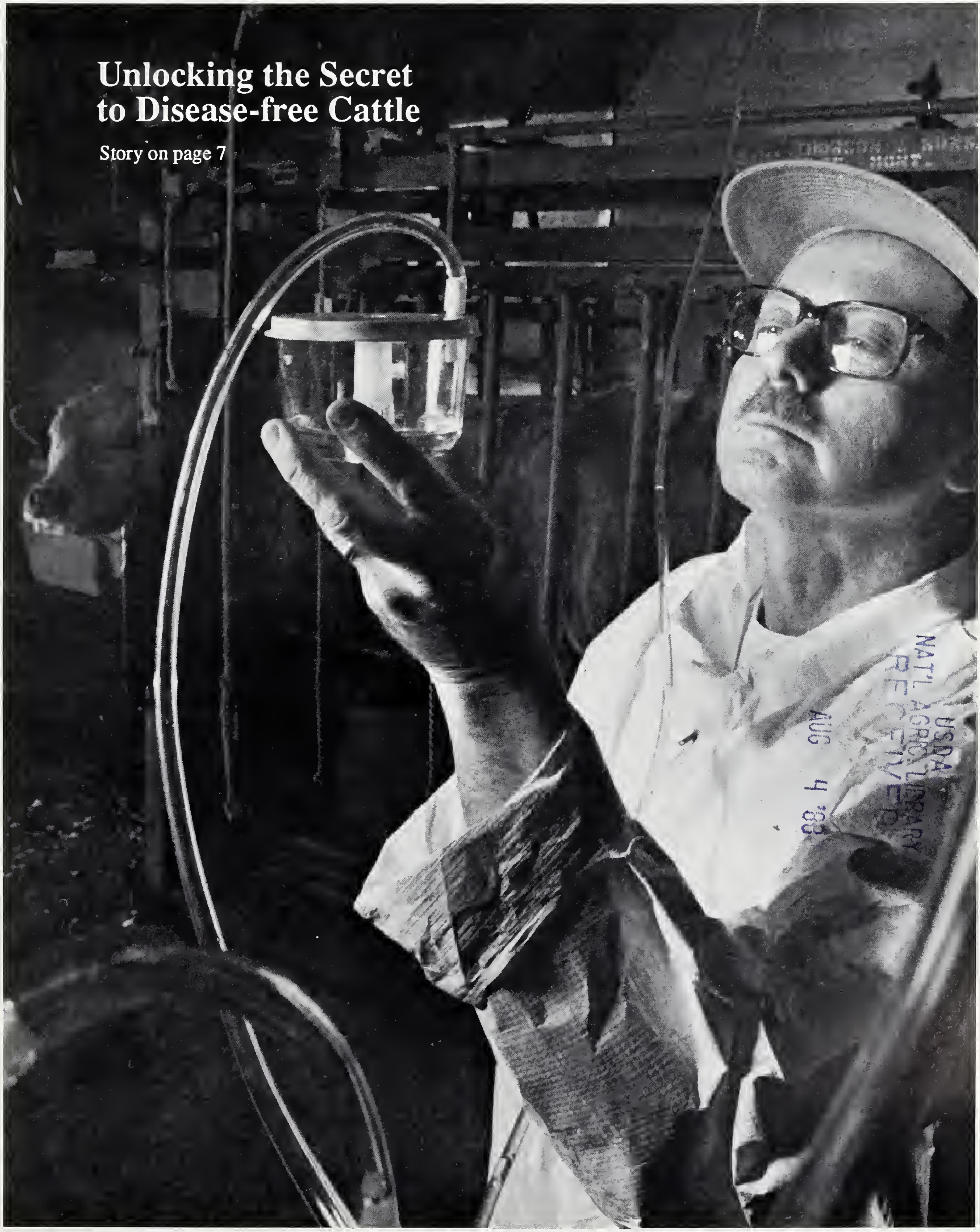

Unlocking the Secret to Disease-free Cattle

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Gene Stock: Locating the Best

With more genetic boosts from foreign animal breeds, U.S. farm animals could be more productive and disease resistant, yield leaner meat, and supply higher quality wool, leather, and other products. Animal scientists eagerly await an important milestone toward that goal—release of a report by the National Academy of Science, due later this year. The report will focus on the need for international cooperation to catalog and preserve farm animals' germplasm—their genetic source material.

Germplasm from plants—seeds and budstock—has long been collected and preserved and has for generations been a contributing factor in the development of new varieties of fruit, vegetables, forage, and ornamental plants. But the idea of similarly pinning down the whereabouts of the animal world's abundant genes is comparatively new.

It's an idea that makes practical sense. Just as any good cook knows that fine cooking starts with the availability of choice ingredients, animal breeders need to pick and choose from the widest possible selection of genes if they are to develop the best livestock. By cataloging available animal gene stock, from the most common to the most obscure, scientific breeders will have access to the ingredients they need without guessing and will be able to more effectively capitalize on genetic variabilities within livestock species.

When the best genetically controlled characteristics have been identified and can be used in breeding programs, results of selective breeding can be spectacular. Consider the successes of the national program to exploit the variability of genes in dairy cattle. Since 1955, milk production per cow in the United States more than doubled, allowing farmers to reduce the number of animals in production by about one half. The result has been a continuing supply of milk and milk products at a reasonable cost to consumers at a time when per cow costs have steadily risen.

Including milk, animal products provide us with 70 percent of our protein, 35 percent of our energy, 80 percent of our calcium, 60 percent of our phosphorus, and other essential vitamins and minerals.

A program to help locate and identify farm animals worldwide with valuable genes would help us not only to produce ample supplies of high-quality food, but could also be used to breed resistance to disease in livestock. As we continue to move ahead with research involving the new tools of biotechnology, we will find even more need for a strong and vital germplasm program.

The topic was highlighted at a 1988 Agricultural Research Service symposium on *Biotic Diversity and Germplasm Preservation: Global Imperatives.* In the words of ARS animal geneticist Keith E. Gregory, "No organized program exists in the United States or other countries to sample, evaluate, and optimally use the genetic diversity in animals that supply food and fiber to the world."

Roger J. Gerrits, who oversees ARS' nationwide research on animal productivity, contends, "An animal genetic resource program must be established to accelerate genetic progress, improve efficiency of production and ensure a continued supply of animal products at a reasonable cost to the consumer. The program would somewhat parallel the decades-old organized effort to preserve the genetic material of plants."

Some known advantages of foreign livestock that await exploitation:

Certain European swine have genes that give them a higher ratio of lean to fat than American breeds have. And many breeds from the People's Republic of China are more prolific than American breeds.

Bos indicus breeds of cattle in eastern and southern Africa, Pakistan, and India have genes for tolerance to heat and nutritional stress that could benefit U.S. beef production systems.

Sheep with genes for more multiple births or for becoming pregnant at any season of the year could benefit U.S. sheep producers. Recent imports such as Finnsheep from Finland, Booroola Merina from Australia, and Romanov sheep from Russia have added genetic diversity to the U.S. flock. Other foreign sheep breeds produce leaner carcasses, such as the Texel that originated in The Netherlands.

Domestically produced mohair comes from a strain of Angora goats in central Texas that descended from a narrow genetic base. Sampling from the much larger and more genetically varied Angoras in Turkey and the Republic of South Africa would broaden the genetic base of U.S. animals.

Today it is more feasible than ever to capitalize on animal genetic diversity because of advances in disease diagnosis, new washing techniques for removing viral pathogens from embryos, and the international transport of frozen embryos. In this month's issue, science writer Vince Mazzola tells us of advances in embryo technology, describing refinements originating in the laboratory that are changing livestock breeding in the United States.—**R.W.**

Agricultural Research

Cover: Robert A. Bellows flushes seven-day-old embryos from a cow treated with a hormone that induces multiple ovulation. Recovered embryos are implanted into surrogate mothers or frozen for later use. Photo by Jack Dykinga. (88BW0902-4)

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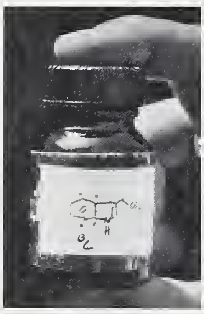

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Agricultural Research Service

Overhead Volts Do Bossy No Harm

Can cattle be safely grazed and confined under high-voltage electrical lines?

"Yes," says an Agricultural Research Service scientist.

That answer came from a 3-year, $1.5 million study in Oregon of what happens to cows staying under or 1,500 feet away from a 500,000-volt power line. This line is one of the most powerful in this country, stretching about 900 miles from a Columbia River dam at The Dalles, Oregon, to Los Angeles.

Since the mid-1970s, many farmers, particularly in the Midwest, have protested that extremely high voltage causes losses in beef production.

In Minnesota, vandals destroyed 10,000 insulators and toppled 16 steel support towers, causing almost $7 million in damage between 1979 and 1986.

In 1984, the Bonneville Power Administration and nine other U.S. and Canadian power companies joined in sponsoring a study through Oregon State University in cooperation with Agricultural Research Service scientists. The study, begun in 1985, was designed to address the concerns of cattle producers everywhere.

"Most producers want to know how many pounds of meat they can sell at market," says ARS rangeland scientist David C. Ganskopp in Burns, Oregon. "They also want to know about anything that will affect the amount of feed and water cattle consume, the number of calves born, cattle pregnancy rates, and calving dates."

According to Ganskopp, "One can hear snapping, buzzing, and popping when standing under the powerlines. Depending on which side of the line you're under, your hair feels as though it's either plastered to your head or standing on end."

Two hundred cows were used in the study. They were separated into groups of 50. Each group had its own feed pen, which was either directly under the powerlines or 1,500 feet away from them.

Twice a week, Ganskopp and coworkers plotted the position of each cow during feeding, afternoon resting, and sleeping. Each month, they weighed the cattle and recorded their activity every 15 minutes for 24 hours and animal position in the feeding pen every hour for 24 hours—96 observations of activity and 24 of position.

The scientists found that although a significant number of animals (4 to 16 percent) moved away from areas directly under the lines during their resting periods, no cattle were physically affected at any time during the study.

The study also showed there were no significant differences in feeding positions between the cows under the powerline and those farther away from it, says Ganskopp. "At night, the cattle were evenly distributed, providing no evidence to suggest they were altering their positions with respect to their selection of bed sites.

"Overall," he says, "results from this study suggest that farmers do not have to make allowances for animal behavior under or near high-voltage powerlines."—By **Howard Sherman, ARS.**

David C. Ganskopp is in USDA-ARS Range and Meadow Forage Management, Star Route 1, 4.51 Highway 205, Burns, OR 97720 (503) 573-2064. ♦

Worm Vs. Worm, Farmers Benefit

A microscopic worm called a nematode could benefit farmers and gardeners by becoming a new biological control for troublesome corn rootworms, says an Agriculture Research Service scientist.

While many parasitic nematodes victimize plants, a few species attack and kill only insects, says entomologist Jan J. Jackson. One nematode, *Steinernema feltiae,* infects rootworm larvae in the soil and releases a bacterium that kills them.

The nematode then turns the larva's body into a sort of mating room where it reproduces and feasts on bacteria and larval tissue. About 10 days later, hundreds of new nematodes emerge in search of more rootworms.

"The worms provide an advantage over chemical controls," Jackson says. "A chemical just sits there and waits for the rootworm to come to it. The nematode doesn't wait."

But there's a problem, so far. Jackson found that the nematodes don't persist underground long enough to eliminate a rootworm population. In one field trial at the ARS' Northern Grain Insects Research Laboratory, Brookings, South Dakota, Jackson applied 100,000 nematodes to the base of each of several corn plants. Root damage was reduced about 11 percent.

Although that isn't an acceptable level for control, he expects further research could lead to protection as good as that from chemical insecticide. A separate test with an insecticide reduced root damage 44 percent.

Rootworms are one of the most serious pests of U.S. corn and can be especially abundant where corn is planted in successive years. The larvae destroy corn roots, and adults feed on the leaves, tassels, and ears.

Jackson says U.S. corn producers annually apply 25 to 30 million pounds of chemical insecticides to battle the rootworm, a practice that costs them nearly $350 million.

Nematodes, on the other hand, could probably be produced at an equivalent price, without the risk of chemical injury to the farmer and environment, he says.

"On the whole, insect-parasitic nematodes have been a poorly studied group, and yet they have much potential as a biological control agent," Jackson says. "But we don't want to overstate our case before we have a dependable control system, one that farmers can rely on."

The next step in the study will be to look at nematode characteristics such as longevity, heat sensitivity, and mobility to identify factors that

limit its persistence. Selective breeding may also be used to strengthen its effectiveness. —By **Matt Bosisio,** ARS.

Jan J. Jackson is with the USDA-ARS Northern Grain Insects Research Laboratory, Brookings, SD 57006 (605) 693-5205. ♦

Sweetpotatoes Make Good French Fries

Are American consumers ready for sweetpotato french fries? Agricultural Research Service scientist William M. Walter Jr., at Raleigh, North Carolina, thinks they are.

Walter says french-frying the sweetpotato may liberate that root vegetable from its traditional place at the holiday dinner table and put it on restaurant menus, right alongside the more popular white potato.

It's already happened at Wolensky's, a Washington bar and grill that sells 100 to 150 pounds of sweetpotato french fries each week, according to chef Kevin Long.

"We serve them with raspberry vinegar, and they're very popular," Long says.

TIM McCABE

Chef Kevin Long with a plate of his popular sweet potato french fries. (88BW0820-34)

Walter, a chemist, is optimistic about sweetpotato fries. His studies show that after 1 year of frozen storage, the cooked fries retained their flavor, texture, appearance, and beta carotene, which the body converts to vitamin A.

"Our tests show that storage stability shouldn't be a problem if industry wants to develop sweetpotato french fries." Walter says. "We've had a lot of calls on this research, and sooner or later the product may be widely available. Today only a limited number of restaurants have them on the menu."

How do they taste? "Imagine sweetpotatoes that have a texture somewhat like french fries," he says. "They taste especially good with vinegar, salt, sugar, or other seasoning."

He hopes the study will spur interest in the sweetpotato, *Ipomoea batatas.* Although it's among the leading vegetable crops worldwide, the sweetpotato has been underused in the United States since it was established in Virginia in the mid-17th century.

Most of the attention, of course, has gone to the Irish, or white, potato. In 1987, the United States produced 38.6 billion pounds of white potatoes, compared to 1.2 billion pounds of sweetpotatoes.

"The sweetpotato's underutilization is unfortunate. After carrots, it's one of the best vegetable sources of beta carotene," Walter says. "People can get their necessary vitamin A by eating sweetpotatoes. White potatoes, by comparison, have little or no vitamin A."

The body converts the orange-colored beta carotene into vitamin A, an essential nutrient for vision; growth; development of bones, teeth, and skin; and other functions. The Recommended Dietary Allowance of vitamin A is 4,000 International Units (IU's) for adult women and 5,000 for men.

About 3.5 ounces (100 grams) of raw sweetpotatoes contain about 8,800 IU's of vitamin A. The same amount of raw carrots has about 11,000 IU's.

The study, conducted in collaboration with North Carolina State University food scientists, also showed that sweetpotatoes maintain their texture better if they are slightly dehydrated before freezing. Jewel and Centennial varieties, two main types grown in the southeast, were used in the study.—By **Sean Adams,** ARS.

William M. Walter Jr., is in USDA-ARS Food Science Research, Box 7624, North Carolina State University, Raleigh, NC 27695-7624 (919) 737-2990. ♦

Nitrogen Losses From Soil Calculated

Concentration of the protective ozone layer in the Earth's upper atmosphere decreased by 2 or 3 percent between 1969 and 1985. Concern that this loss will continue has spurred scientists into learning more about how atmospheric gases react with each other.

Ozone, a 3-atom molecule of oxygen, filters out much of the ultraviolet radiation from the sun. This radiation increases the chances of cataracts and skin cancers. Near ground level, ozone is a major component in smog and aggravates breathing problems.

Commercially manufactured chlorofluorocarbons are alleged to be major culprits in causing the decreasing levels of ozone in the upper atmosphere, but two gases that naturally escape from the soil are also involved.

Just how much of the two—nitric oxide and nitrous oxide—escape and in what proportion is the subject of an Agricultural Research Service study at Fort Collins, Colorado.

Nitrous oxide is an inert gas on the Earth's surface but eventually ends up in the upper atmosphere where it destroys ozone. Concentration of this gas in the atmosphere has increased by 2 to 3 percent every 10 years since 1950.

Continued on page 6.

Nitric oxide remains at the surface and is a catalyst in ozone production. Both gases contain one oxygen atom, but nitric oxide has only one nitrogen atom compared to nitrous oxide's two.

So far scientists have only been able to estimate nitrogen losses and releases from chemical reactions. Of the 13 to 15 million tons of nitrous oxide produced each year, about half comes from tropical and subtropical forests and woodlands. Fertilized fields contribute about 1 million tons annually and burning of fossil fuels about 2 to 4 million.

Estimates of nitric oxide production range from 25 to 99 million tons per year, with fossil fuel and forest burning responsible for nearly three-fourths of the total. Farming has not been included in these estimates but microbial activity in soils contributes an estimated 20 percent of the total.

"While the value of nitrogen that naturally escapes from cropland may be only a few dollars per 100 acres, we want to learn how to manage the land so even less is lost," says soil scientist Gordon L. Hutchinson.

"Surprisingly, annual nitric oxide losses were larger from native grasslands and conifer forests than from most cultivated fields. The only time we measured more from cultivated land was immediately after fertilization," he says.

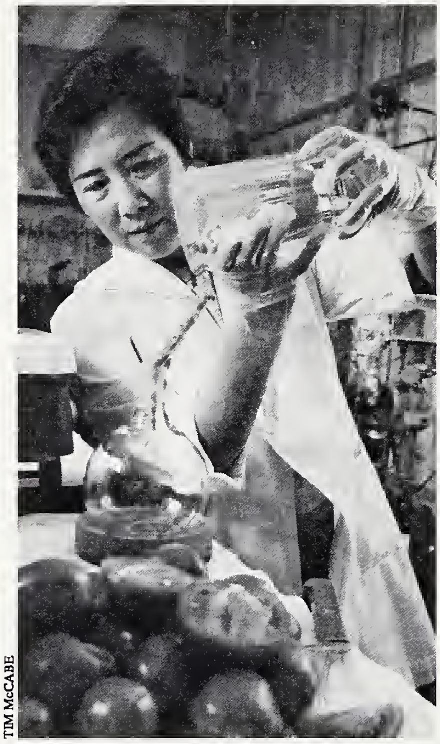
The scientists are now expanding their studies to learn how different tillage practices, soil types, and climates affect release of nitrogen gases.—By **Dennis Senft,** ARS.

Gordon L. Hutchinson is in Soil-Plant-Nutrient Research, 301 S. Howes, P.O. Box E, Fort Collins, CO 80522 (303) 482-5733. ♦

Stop—Don't Slice That Tomato!

Unless, of course, you plan to eat it right away.

An Agricultural Research Service scientist in Albany, California, says the natural chemicals in vine-ripened tomatoes that give them a pleasing aroma are formed when the tomato is cut. About 3 minutes after you slice into the fruit, the aroma starts to fade away.



TIM McCABE

In Albany, California, physical science technician Louisa Ling prepares commercially grown tomatoes for testing to determine their flavor and aroma constituents. (0787X733-16)

"That's why the tomato you put in your sandwich when you're packing your lunch in the morning doesn't really have that much fresh tomato flavor by noon," says chemist Ronald G. Buttery.

Wait until the last minute to slice a tomato, he recommends. And as for the advice Mom gave about not putting tomatoes in the refrigerator, Buttery says she's right. He has new information to prove it.

In his experiments, tomatoes kept in the refrigerator—a common practice at home and at supermarket produce rooms—had less of the aroma-imparting chemical (Z)-3-hexenal than did tomatoes kept at room temperature.

In fact, even tomatoes that were originally picked and shipped long before they were ripe but were later allowed to ripen naturally at room temperature, had about the same amount of key aroma chemicals as freshly picked, vine-ripened tomatoes.

Buttery says scientists have known for more than 15 years about many of the tomato chemicals that play a key role in aroma—and thus flavor. But they have been unable to precisely measure the amounts of these compounds. Researchers in the 1970's, for example, were able to show that refrigeration lowers the total level of aroma-imparting chemicals but weren't able to pinpoint changes in the amounts of individual chemicals.

"The chemicals that give the fresh tomato flavor change too rapidly once the tomato is opened," Buttery says.

Along with colleagues at the ARS Western Regional Research Center, he has developed a new tactic to temporarily halt the enzymes that initiate the chemical changes.

And once he could precisely measure each of the aroma-linked chemicals, Buttery could track their progress when tomatoes were cut open, or were popped into the refrigerator for storage.

"We're looking at aroma chemicals because aroma is the most important part of flavor, as most people realize when they get a cold," he says. "If your nose is blocked, the aroma chemicals in the food you're chewing can't stimulate the olfactory senses and you can't really taste the flavor of what you're eating."

His tomato experiments are aimed at helping breeders develop new varieties of commercial tomatoes that would have the rich, sweet taste of a vine-ripened harvest, yet would be hardy enough to withstand the rigors of the trip from grower to grocer.—By **Marcia Wood,** ARS.

Ronald G. Buttery is in USDA-ARS Food Quality Research, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710 (415) 559-5667. ♦

High Tech Lends Helping Hand to Cattle Producers

Giving shots, pills, and treatments for diseases to 40 million calves a year is a lot of work. Expensive too. It is estimated that when all disease-related losses are added in, producers spend around $9 billion on cattle health.

So it's no wonder that a beef or milk producer gets a little starry-eyed when scientists start talking about cattle with natural immunity to bluetongue, stomach worm, or brucellosis. Disease resistance would be part of the cattle's genetic makeup and, ideally, could be tailored for specific problems in different parts of the country.

"Our first attempt at implanting split [cattle] embryos... produced three sets of identical twins...."

Louis C. Gasbarre, Agricultural Research Service

BOB BJORK

At Beltsville, Maryland, twin Angus cows resulting from split-embryo transplants are part of Louis C. Gasbarre's project to speed research in disease resistance by breeding cattle with identical genetic makeup. (1187X1265-32)

But on learning how much time it will take to do this with conventional crossbreeding, the cattle producer comes crashing back to reality. Immunity doesn't breed in easily, because it's not controlled by a single gene. Instead, immunity involves possibly hundreds or thousands of genes. Compounding the breeder's perplexity, no one really knows how many or even what the total gene count for cattle may be. So even the most optimistic expect it will take 40 to 100 years to reach the first milestone on the road toward inbred immunity—a herd of cattle with nearly identical immunity genes.

But this milestone may be a lot closer than expected, thanks to the work of microbiologist Louis C. Gasbarre of the Agricultural Research Service's Beltsville, Maryland, research center.

He's taking a high-tech shortcut that may shorten the wait to as little as 6 years using proven techniques of embryo splitting and implanting along with innovative immunological research.

Briefly, the plan is to take a fertilized egg from a cow within 7 days after it's fertilized and split it in two. The two pieces, still very much alive and with identical genes, can then be reinserted into two other cows. When these surrogate moms give birth, it's the beginnings of a herd with identical major histocompatibility (immunity) genes.

Transferring cattle embryos was first done successfully by the University of Wisconsin's Elwyn Willett in 1951. The technique is most often used to produce more than one calf from a purebred cow from a single artificial insemination. Embryo transfers and the more difficult splitting and transfer start by injecting a cow with a hormone. This stimulates the cow to superovulate—producing up to 18 eggs. She is then artificially inseminated, and a week later the embryos are harmlessly flushed from her uterus.

They are implanted into surrogate mothers—usually of a less valuable breed than the true parents. In this way, breeders produce a relatively large number of offspring from one insemination.

Gasbarre says, "Our first attempt at implanting split embryos in the spring of 1985 produced three sets of identical twins—six heifers—plus a single bull calf whose twin died."

One of the sets of females and the remaining single male—have the same parents. Tests have shown that through chance they have inherited the same major histocompatibility genes.

When the sisters reached puberty, Gasbarre injected them with a hormone, prostaglandin F2 alpha, causing them to ovulate several eggs apiece. They were bred to the surviving bull twin in November 1987. Nine embryos were recovered when the heifers' uteruses were flushed.

Eight of these were implanted in surrogate mothers immediately and one, for lack of an available surrogate, was frozen. When a surrogate did become available the following March, the embryo was thawed and implanted.

According to the laws of genetics, 25 percent of the offspring should have identical genes for immunity. If this works, especially if male and female offspring have those genes, it will be the first time scientists will have access to

cattle with a complete set of identical genes for immunity.

The significance? Immunologically, such a herd of cattle could be treated as though it were a single individual. An organ could be transplanted from one to another without the rejection that happens between animals with different immunity genes, Gasbarre says. Or in more practical terms from a research standpoint: if 10 animals are exposed to a disease or an experimental drug, they should all react the same. Variables between animals due to genetic differences could be ruled out.

Gasbarre readily admits that cattle with built-in immunity to important diseases may still be a long way off. "In fact," he says, "we're only just beginning to identify the actual genes that control resistance." But he envisions a time when there will be a catalog of the cattle available with various combinations of known genes responsible for immunity to diseases. Breeders could choose the animals with genes to suit their purposes—a rancher or milk producer could select cattle resistant to diseases prevalent in his or her area.

One more plus for embryos: they can be frozen and later revived. To prepare them for storage or shipping, embryos are placed in tubes containing phosphate-buffered saline solution and glycerine. They are then cooled at 1°C a minute or less. Slow freezing and the solution prevent water from crystalizing and causing the cells to burst. The tubes are stored in liquid nitrogen at -196°C (-321°F). Theoretically, the embryos can stay in suspended animation for hundreds of years without damage.

Dozens of frozen embryos can be transported easily in a test tube. By comparison, a single cow, bull, or calf costs about $3,500 to import into this country and has to stay in quarantine from 1 to 6 months. Most of this could be saved, particularly if the quarantine to check for diseases could be eliminated.

Gene Stock in the Deep Freeze

Another advantage to frozen embryos is that the genetic makeup of a specific animal can be preserved beyond the animal's lifetime. During a long-term breeding program, embryos could be periodically thawed, implanted, and grown in a surrogate as a point of reference to see what improvements have been achieved through breeding.

There's an added bonus, says ARS' Robert A. Bellows at the Fort Keogh, Montana, research facility. "These frozen embryos may be a source of genes that would otherwise no longer be available to farmers, breeders, and scientists in the future."

Embryo Washing

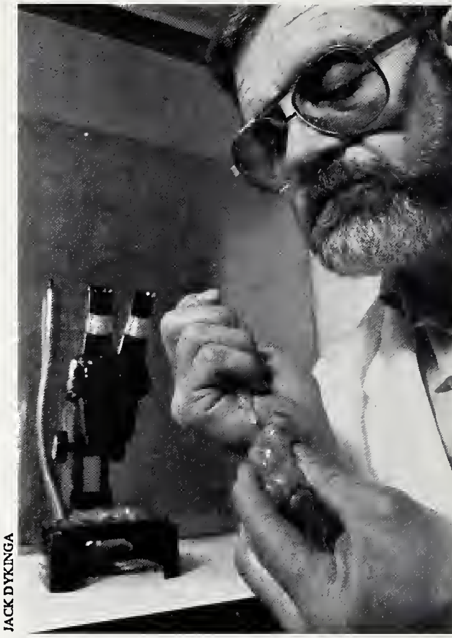
What about the possibility of avoiding quarantine requirements for cattle imports? New technology could bring about such a change in policy, says animal physiologist Sherrill E. Echternkamp. "Embryo washing offers a chance for disease-free offspring from infected parents."

Echternkamp, reporting on tests at ARS' Meat Animal Research Center, Clay Center, Nebraska, says, "Sixty cows were intentionally infected with bluetongue disease, superovulated, and artificially inseminated; then the embryos flushed out. Before implantation into surrogate mothers, the embryos were washed in successive rinses of buffered saline solutions to remove any possible virus on their surfaces. Out of 237 implantations, 94 calves were born. So far, all of the calves and surrogate mothers have proved bluetongue-free."

Scientists at ARS' Plum Island Animal Disease Center reported similar results when they obtained foot-and-mouth-disease-free calves from infected donor cattle using the same embryo transplant techniques.

Bluetongue is a disease that affects ruminant animals. Although cattle are the major carriers of the disease, they don't suffer noticeable symptoms; it's sheep that bear the brunt of the disease: mouth sores, lameness, abortion, birth defects, and death. But bluetongue is an infection which readily travels from cattle to sheep via gnats. This explains the policies of countries that don't have bluetongue disease: to safeguard their sheep, they prohibit the importation of U.S. cattle.

Potentially, a $30 million overseas market exists for U.S. cattle, semen, embryos, fresh meat, and biologicals using cattle serum, blood, and other nonsterilizable components if cattle certified bluetongue-free were available. At least part of that market could be opened up through embryo washing.



JACK DYKINGA

The United States has import prohibitions of its own. We ban livestock imports from countries where foot-and-mouth disease can be found. Embryos may be legally imported only from countries designated foot-and-mouth disease free, such as England, New Zealand, Japan, Canada, and Australia.

This could change if tests affirm the safety of embryo transport from coun-

BRUCE FRITZ

Above: This proud mother has produced three sets of twins in five births. Producing twins can increase the efficiency of beef production by 20-30 percent. (0785X639-13)

Above, left: Animal physiologist Robert B. Staigmiller extracts eggs from the ovary of a cow treated with folicular stimulating hormone in preparation for laboratory fertilization. (88BW0897-35A)

Left: White irregular shapes within this highly magnified uterine cell are a secretion necessary for embryo survival and growth during the first 15 to 16 days of a cow's pregnancy. Electron transmission micrograph by Michael T. Zavy. (88BW1186)

tries with diseases foreign to the United States, says Charles A. Mebus, reseach leader of the pathobiology section at ARS' Plum Island Animal Disease Center. Plum Island, located about 6 miles offshore of Long Island, New York, is the only U.S. location where study of foot-and-mouth disease is permitted.

A Stop to Embryo Death

A major reproductive problem in cattle is embryo death, costing producers an estimated $1 billion in revenue lost from calf sales. Animal physiologist Michael T. Zavy in ARS' Forage and Livestock Research Laboratory at El Reno, Oklahoma, hopes to find out why some 20 to 40 out of every 100 embryos fail to survive to produce a calf.

Zavy points out that an embryo is not passive. It produces hormones that tell the cow's body that it is pregnant. The cow's body in turn directs the lining of the uterus (endometrium) to produce proteins and other substances that control embryonic growth.

He theorizes that "an embryo dies because in the critical 15-16 days after fertilization, right before it starts to elongate and become attached to the uterus, for some reason it is 'out of sync' with the endometrium." In lab tests, out of sync embryos cultured with endometrial cells were brought back into synchrony with the addition of hormones and steroids.

"We don't know yet if infertility can be overcome with these treatments," says Zavy. "But we hope so. Infertile cows are usually slaughtered."

Controlling Calf Size

Calving difficulties, principally from oversized calves, cost ranchers about $300-$500 million a year. Research shows that besides the obvious factors of the mother's age and growth potential of the bull, conditions inside the cow's uterus have an important role in determining the weight of the calf at birth.

To examine what conditions in the uterus affect the birth weight of calves, embryos from the same mother are implanted into surrogate Brahman and Hereford mothers. Various measurements of fetal growth; levels of the hormones estrogen, progesterone, and relaxin; and placental development and attachments are determined during different stages of gestation.

Brahman cows are used as a sort of yardstick to measure the Hereford cows against because the Brahmans somehow control the birth size of calves, resulting in fewer calving problems, Bellows says.

To increase the number of embryos available for freezing and various tests, animal physiologist Robert B. Staigmiller, a colleague of Bellows at Fort Keogh, surgically removes the ovaries from cows and fertilizes the eggs they contain in a test tube. These embryos are implanted in female sheep or rabbits and allowed to develop for 7 days, then frozen for future use. The technique will be refined and used as a basis for developing improved methods for large scale production of embryos.

Twins Improve Production

Each year, a cow that has a single calf produces about 70 percent of her body weight in marketable offspring. By comparison, a sow with her litters of pigs, turns out 8 times her body weight in market hogs, and a meat hen produces 70 times her body weight in broilers.

A cow that has twins almost doubles her output. Of course, growth rates and feed efficiency enter into the profitability equation. "But considering all the costs, cows producing twins increase the efficiency of beef production by 20 to 30 percent," says retired ARS animal geneticist Gordon E. Dickerson.

Increasing the twinning rate is another facet of reproduction that Bellows is working on at Fort Keogh. Although natural twins occur only about once in every 100 births in cattle, Dickerson explains that cattle are physically well prepared to bear twins.

He explains that a cow's uterus is shaped something like the letter Y. During most pregnancies, only one horn of the Y receives an embryo, so he implants another one in the other horn. "For some reason, not yet known, many of the implanted embryos don't live. Still, with this technique we have been able to increase twinning in our cattle by 10 to 15 percent," Bellows says.

Another ARS scientist, Keith E. Gregory, at Clay Center, Nebraska, is building a herd that is genetically more likely to twin than normal herds. Using embryo transfers to speed up the breeding process, he has achieved a natural twinning rate of 6 to 8 percent through selective breeding.—By **Vince Mazzola,** ARS.

Sherrill E. Echternkamp is in the USDA-ARS Reproduction Research Laboratory, Room 258, and Keith E. Gregory is in the Genetics and Breeding Research Laboratory, Room 203, Bldg. 1, Roman L. Hruska Meat Research Center, Clay Center, NE 68933 (402) 762-3241. Charles A. Mebus is in USDA-ARS Pathobiological Research Laboratory, Plum Island Animal Disease Center, Greenport, NY 11944 (516) 323-2500. Michael T. Zavy is in the USDA-ARS Forage and Livestock Research Laboratory, P.O. Box 1199, El Reno, OK 73036 (405) 262-5291. Robert A. Bellows and Robert B. Staigmiller are at the Fort Keogh Livestock and Range Research Facility, Miles City, MT 59301 (406) 232-4970. Louis C. Gasbarre is in the USDA-ARS Helminthic Diseases Laboratory, Room 2, Bldg. 1002, and Lawrence A. Johnson is in the Reproduction Laboratory, Room 22, Bldg. 200, Beltsville MD 20705 (301) 344-2509. ♦

Sperm:
The Other Half of the Reproduction Equation

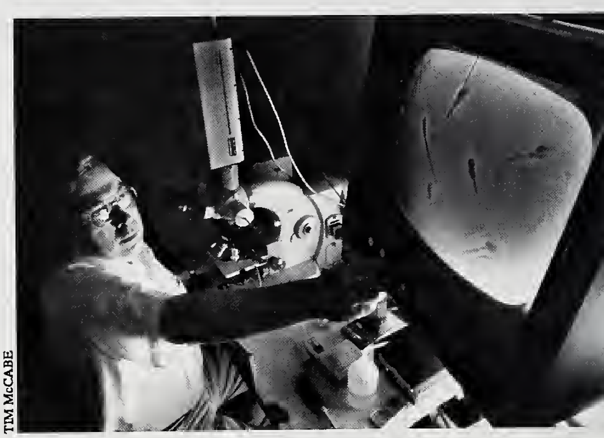
Although not as far advanced as embryo manipulation, sperm cell work is also getting the high tech treatment.

At ARS' Beltsville, Maryland, Agricultural Research Center, animal physiologist Lawrence A. Johnson has developed a laser-based system that in conjunction with a fluorescent dye, sorts out X-chromosome-bearing sperm, for female offspring from Y-chromosome-bearing sperm for male offspring. The technique works by detecting differing amounts of DNA or genetic molecules in X and Y sperm cells.

Since X chromosomes have more DNA than Y chromosomes, they glow more brightly as they drop through a laser beam. A cell counter connected to a computer records the amount of fluorescence per sperm cell *[see* Agricultural Research, *August 1985, pp. 6 and 7]*.

Depending on a sperm cell's brightness, it is given a weak positive or negative charge. The sperm then falls through an electrical field that pulls the positive- and negative-charged sperm heads away from each other and into different containers.

"We can now sort out female- and male-producing sperm for cattle, swine, and sheep. Unfortunately, sperm cells sorted this way cannot be used for routine artificial insemination because they have to have their tails and membranes removed for dyeing. Thus, it's impossible for them to move and fertilize an egg on their own. In any case, sorting by DNA content is too time-consuming to be practical for livestock breeders," says Johnson.


TIM McCABE

Animal physiologist Lawrence Johnson watches male sperm cells to evaluate their motility, a procedure that precedes laser X-Y sperm separation. (88BW0873-3)

He says their work is laying the foundation for a sex-selection technique that will increase the efficiency of the livestock industry.

"If farmers could select the sex of their animals, it would probably mean a savings of several hundred million dollars a year," he says. For example, beef producers want mainly male cattle because they gain weight faster than females and usually command a higher price at slaughter time. Dairy farmers could produce female calves for herd replacement, thus reducing the time and expense of raising bull calves for slaughter.—**V.M.**

Better Bread Through Biotechnology?

Bread, muffins, cakes, cookies, pasta and other tasty products made from the flour of American-grown wheat might someday be even better tasting—and more nutritious—thanks to genetic engineering and an unconventional use of baker's yeast.

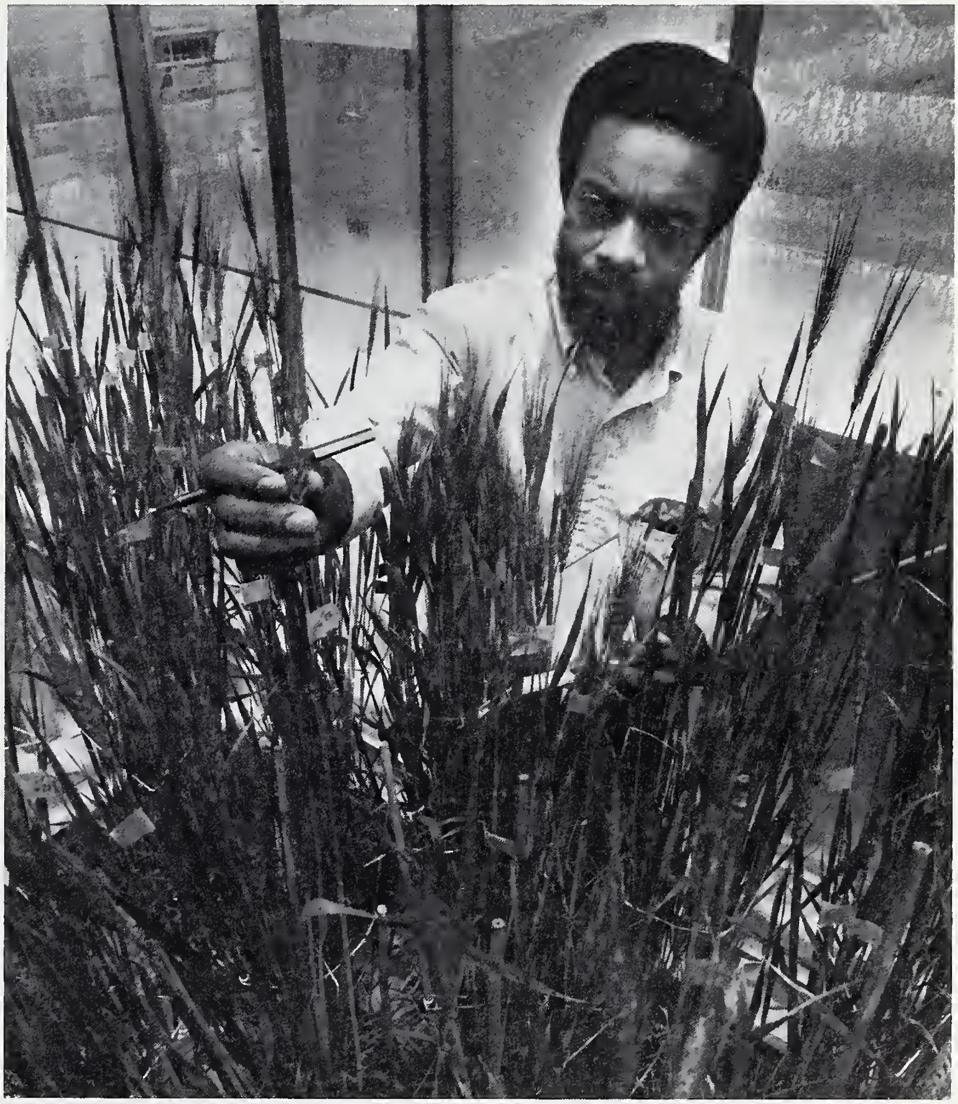
A team of Agricultural Research Service and University of Hawaii scientists has genetically modified a yeast to make it capable of producing a major protein of wheat flour, known as a gliadin. That protein is related to baking quality, says Frank C. Greene of the ARS' Plant Development Quality Research Unit in Albany, California.

The yeast system makes it easier for the researchers to examine and experiment with the gliadin than if the protein were still locked up with others inside the wheat kernel.

Greene and ARS colleagues began by isolating a gene that directs the wheat kernel to manufacture the gliadin protein. They then collaborated with the Hawaii group, led by John I. Stiles in Honolulu, to insert this gene into cells of a strain of the yeast *Saccharomyces cerevisiae*. The yeast cells, following instructions encoded in the wheat gene, now produce the protein, says Greene.

The Federal Government is currently applying for a patent for this work.

More recently, the ARS scientists, in collaboration with researchers in England, isolated two glutenin genes, thought by some experts to be even more important than the gliadins in determining superior baking quality.



TIM McCABE

In his Albany, California greenhouse, ARS chemist Frank Greene checks developmental state of wheat used in grain protein studies. (0787X711-5)

Wheat is the only grain in the world that can be made into a dough that rises and produces light, fluffy baked foods, according to Greene. For millers and bakers, baking quality of the wheats they use describes such features as lightness, crumb composition, and texture.

Why does baking quality need to be improved? "Doughs and batters made from the flour of today's wheat produce excellent products—but millers and bakers believe that they could be even better," Greene explains. An example: they want wheat that contains a larger quantity of the proteins that enhance baking quality, he says.

Just as genetic engineering of wheat might result in superior baking quality, the technology might also lead to improved nutritional value. "We want wheat proteins to contain more of certain amino acids, especially lysine and threonine," Greene says. Of the amino acids in wheat flour, those two are often in the shortest supply. Both are essential, in that we must have them in order to stay healthy.—**Marcia Wood, ARS.**

Frank C. Greene is in USDA-ARS Plant Development Quality Research, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710 (415) 559-5614. ♦

Wonderful World of Yeast

Bake a loaf of bread or drink a bottle of beer and you've experienced two of the most common uses of yeast. But for all the familiarity, there is more to this mysterious microorganism than meets the eye. In your yard and garden, in your refrigerator, even at times on your skin, yeasts are busily at work, multiplying by the millions.

That is one of the amazing facets of yeasts that have awed scientists for years. Hundreds of strains and species of yeast are at large, and many are individualists. Yet, taken as a whole, they are a regiment of versatile and disciplined soldiers out to convert the world into usable energy.

"What we've done in the laboratory is harness the potential of yeast that already exists in nature," says Cletus P. Kurtzman, Agricultural Research Service microbiologist. "For thousands of years, humans have put yeast to a variety of different uses, from fermenting grapes to baking leavened bread. And even though those uses themselves are terribly important still, the potential future uses of yeast are incredible."

Kurtzman watches over 77,000 strains of yeast, bacteria, and molds at ARS' Northern Regional Research Center in Peoria, Illinois. It is the largest such collection in the world and includes 14,200 different strains of yeast and 600 individual species.

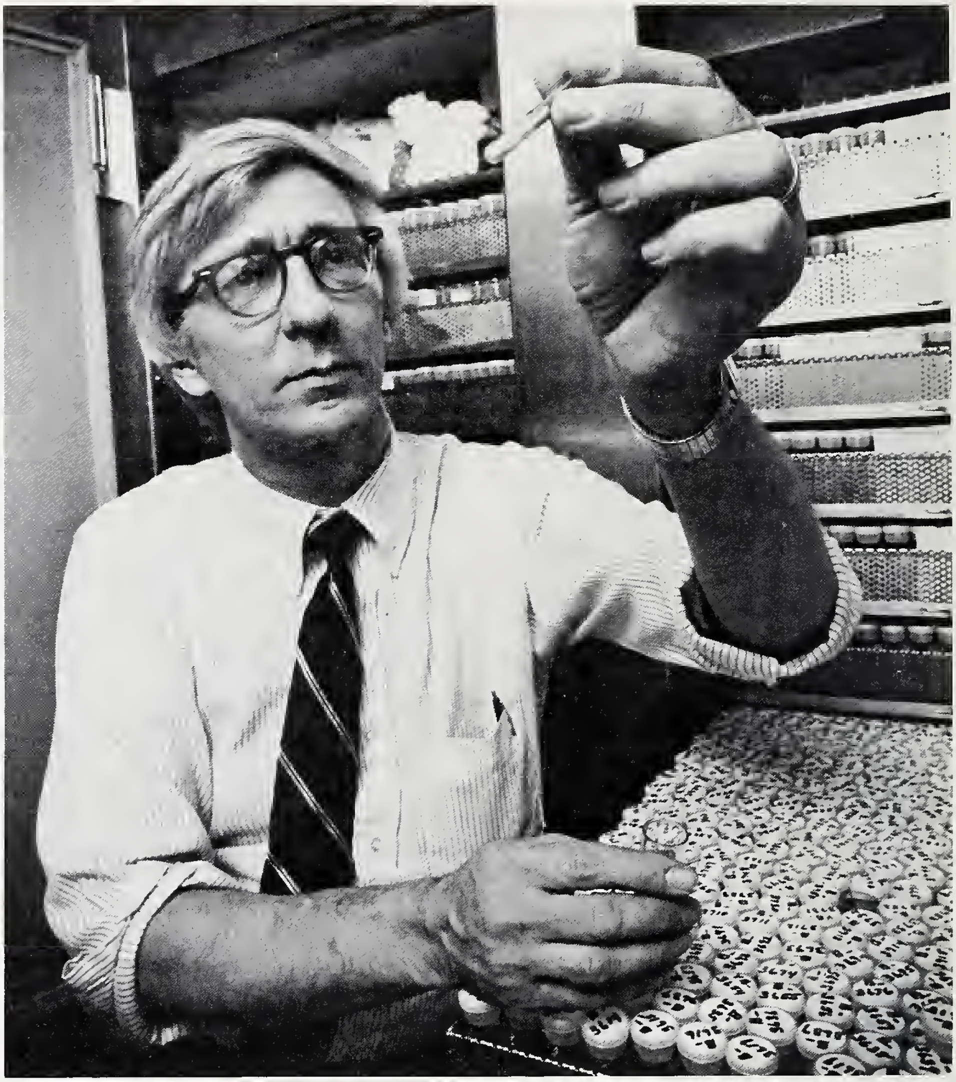
Among them is baker's yeast, *Saccharomyces cerevisiae,* which continues to be the workhorse of yeast studies, and *S. carlsbergensis,* a yeast long used for brewing.

In the food industry, yeasts are often used in producing soups, soy sauce, baby food, grape juice, wine, cider, and other products. It is used to break down lactose and starch, to treat industrial and agricultural wastes, and to produce vitamin supplements and ethanol fuel.

In the Orient, yeasts are used to ferment rice and cassava, both of which are turned into popular sugary desserts.

Some of the uses are the result of work done at the USDA's Agricultural Research Service to make yeast more productive and accessible to agriculture.

Kurtzman says yeasts are traditionally used to turn sugars into forms of energy.



Microbiologist Cletus Kurtzman is curator of the world's largest collection of yeasts. (1284X1847- 34A)

In bread, for example, yeast converts sugar into carbon dioxide, which makes the bread rise, and alcohol, which evaporates during baking.

Yeasts as sources of enzymes and specialty chemicals are promising concepts currently under study. Some of these and other possibilities for yeast that scientists envision in the years ahead, though, are strictly for the imaginative. They include:

• Zeroing in on blood clots. Scientists believe that yeast can be used to produce a fast-acting enzyme that, when injected in a vein, will rush to the site of a blood clot and eat it away. Such a procedure could lessen the damage caused by heart attacks.

• Cheaper citric acid. Yeast could be used to produce citric acid in a process that would be less expensive and more productive than commercial methods now used, which employ molds. Citric acid is added to carbonated beverages, syrup, food, and pharmaceuticals.

• Recombinant DNA technology. "That's the big one," Kurtzman says. "That's the most spectacular future use of yeast now being studied because of the convenience of creating large numbers of biochemicals inexpensively

Photomicrograph (magnified about 200 times) of an unusual yeast, *Pachysolen tannaphilus,* in the ARS collection at the Northern Regional Research Center in Peoria, Illinois, that converts xylose (wood sugar) directly into ethyl alcohol. It is estimated that yeasts like *Pachysolen* could produce 4 billion gallons of fuel alcohol from crop wastes in the United States. (PN-6850)

Animalcules?

When the descriptions and drawings of tiny "animalcules" arrived at the Royal Society of London in 1680, no one knew what to make of them. Antonie van Leeuwenhoek, the Dutch inventor of the single-lens microscope, claimed they were microorganisms he found living in his beer.

By 1837, those "animalcules" were being described by some as living fermenters that could consume sugar and excrete alcohol and carbon dioxide. The theory was lambasted by numerous chemists who preferred to explain fermentation as a chemical reaction rather than the activities of living cells.

It wasn't until Louis Pasteur's studies in 1866, in which he verified the cellular action in fermentation, that a modern understanding of yeasts was established and refined. Through his efforts, and the work of the other pioneers of microbiology, study of yeasts was catapulted into the highly respected ranks of scientific inquiry.

The time of the first human use of yeast, though, remains a mystery. From excavations at the Egyptian city of Thebes, archeologists have uncovered evidence that yeast was commonly in use in bread-baking and brewing as early as 2,000 B.C. Models of a bakery and brewery and sediment from a beer jar uncovered from a tomb suggest that yeast may have been in general use even earlier.—**M.B.**

through fermentation." Genetic transfer is already possible using yeast as a vehicle. Still to be developed, though, are improved methods of introducing genes from one yeast cell to another to provide stronger or new characteristics to that yeast.

• Drug applications. Yeast-manufactured hormones could someday be developed to regulate metabolism for weight control. Yeasts might also be used to produce an enzyme to stimulate hair growth or reject disease-causing agents not presently rejected by the body.

• Flavor enhancers. Yeasts could be engineered to impart specific flavors to certain foods, more so than has been done in the past. A potato, for example, could be made to taste like cheese.

But not all yeast is good yeast, and some good yeasts can perform in harmful ways. A yeast found in pigeon droppings can enter the human nervous system and cause death. At least 50 percent of those who are infected by this particular yeast, called *Cryptococcus neoformans,* die from it.

Eating too much yeast may lead to gout or symptoms of gout because of uric acid produced when certain yeast products are metabolized by the body.

Thrush is an infection of the mouth caused by the yeast *Candida albicans.* It is characterized by formation of white patches and ulcers in the mouth lining and most often infects infants and young children.

The same yeast is responsible for vaginitis, a painful yeast infection that afflicts thousands of women. "No one can really explain it," Kurtzman says. "Your metabolism changes, your temperature drops, or something else happens to the body and this yeast just takes off."

For the most part, however, scientists have found yeast to be a helpful, flexible, and efficient assistant in producing products and processes that enhance living.

"In fact, if we didn't have yeast," says C.W. Hesseltine, an ARS microbiologist now retired, "organic material would just pile up around us. It wouldn't decompose. We would just die from smothering in our own waste."

Chances of that happening, though, are slim, he added. Whether in the air, on the skin or in the lab, yeasts are here to stay, mysterious toilers in the biological world.—By **Matt Bosisio, ARS.**

Cletus Kurtzman is at the USDA-ARS Microbiological Properties Research Unit, Northern Regional Research Center, 1815 North University St., Peoria, IL 61604 (309) 685-4011. ♦

Plant Hormones Could Help Shape Tomorrow's Crops

Crops might be redesigned to use their own hormones better, according to an Agricultural Research Service scientist, now that he has found a way to measure plant hormone levels more accurately.

"We've made a quantum jump in accuracy," says Jerry D. Cohen, a biochemist in Beltsville, Maryland.

"We only need about one percent of the plant material previously required for hormone measurements," he says. "That means we can zero-in on specific parts of a plant."

Knowing how much of a hormone is reaching the leaves, buds, or roots of a plant, says Cohen, could help bring about the genetic engineering and breeding of crops for size, shape, budding, ripeness, and other characteristics highly dependent on hormone production.

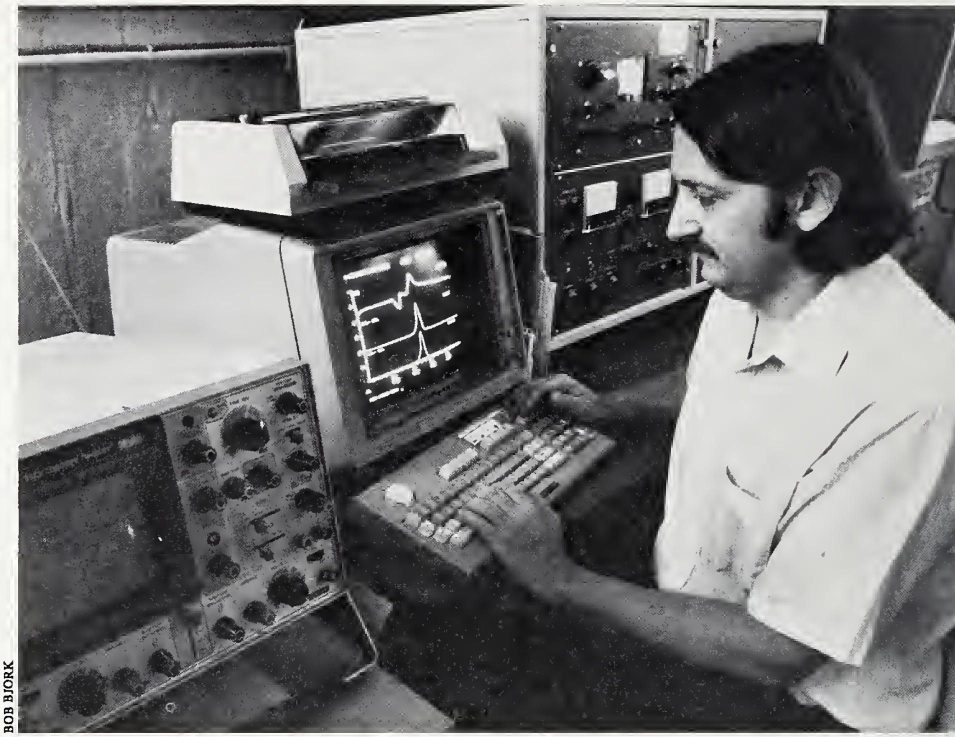
"It may only be the first step," he adds, "but it's a big one towards the day we can improve the genetic code for hormone production in crops."

Somewhat like their counterparts in humans and animals, hormones in plants are chemicals made by plants to stimulate their own growth and development. Scientists have analyzed the molecular structures of numerous plant hormones and have even synthesized some of them, but until now they have been unable to determine exactly how much of a given hormone was in any particular part of the plant.

"That's because they exist in such small amounts," explains Cohen. "You could find maybe 1 ounce of hormone in 40 tons of plant tissue, if you look hard enough. And you have to be sure that you've found the real thing. There's a lot of molecular garbage to sort through—a lot of molecules that appear to be hormones but aren't."

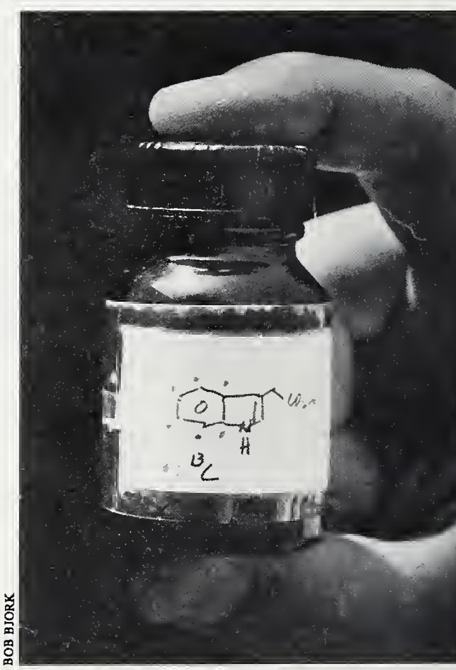
Cohen began his research on plant hormones while a graduate student at Michigan State University. Some of the measurement techniques he developed there are now being used in psychiatric and forensic studies that link schizophrenic and psychotic behavior to abnormal hormone levels in the brain.

"Hormone levels in humans and animals are a lot higher than in plants," he says, "and can be measured more easily. We were on the right track at Michigan, but our methods simply weren't precise enough."



BOB BJORK

Above: Peaks and valleys on the screen of a mass spectrometer enable biochemist Jerry Cohen to isolate and measure the plant hormone IAA. (88BW0949-24A)



BOB BJORK

Right: Contained in this jar is the world's known supply of specially modified IAA, containing carbon-13, a radioisotope. The mixture is used to determine plant hormone quantities. (88BW1011-28A)

At ARS, Cohen perfected his method for measuring plant hormone quantities while investigating a hormone known as indole-3-acetic acid, or IAA for short. IAA was the first plant hormone to be identified as such and is one of five major groups of hormones that are found in all plants.

To determine IAA levels in plant tissue, Cohen thoroughly mixes the tissue with a known quantity of specially modified IAA containing a carbon isotope—a form of carbon having a greater molecular weight than normal. He then places a sample of the mixture in a mass spectrometer, which enables him to distinguish the natural IAA from the heavy version and compare their respective quantities. Once the ratio between the two kinds of hormone in the sample is established, the amount of natural IAA in the whole mixture can be determined as well.

"The use of carbon isotopes and a mass spectrometer makes the process

work," says Cohen. "Although we've been looking at IAA almost exclusively, there is no reason the same techniques, possibly with other kinds of isotopes, won't work for other hormones as well."

At present, hormonelike chemicals marketed as growth inhibitors and growth promoters are sprayed on crops to get them to achieve a desired size, shape, and ripeness by harvest time. But these external treatments can be costly and don't always work as expected. They have to be repeated each season, and some crops seem to sense that their development is being influenced unnaturally and adjust their own hormone production accordingly.

"Changing the genetic code could be more effective," says Cohen, "because it gives the new hormone balances hereditary status. They become a natural part of the plant and show up in future generations."—By **Steve Miller,** ARS.

Jerry D. Cohen is in the USDA-ARS Plant Hormone Laboratory, Room 30, Bldg. 050, BARC-West, Beltsville, MD 20705 (301) 344-3632. ♦

PATENTS

Making Travel Easier for Nematodes

Tiny, transparent worms known as beneficial nematodes can force their way inside a destructive beetle, borer, gnat, grub, weevil, fruit fly, or rootworm and stop it dead.

It takes them less than 3 days to kill many of these enemies of home, garden, or farm.

Now there's a new, convenient, and comparatively inexpensive way to move hundreds of millions of the helpful worms from the laboratory vats or vials in which they are reared to the backyards, fields, and orchards where they're needed, says ARS entomologist James E. Lindegren at Fresno, California.

One of the biggest obstacles blocking widespread use of nematodes as biological pest controls has been the complications in shipping them from one place to another.

"You have to make sure they're in the proper stage of their life cycle, and that they have the oxygen and moisture they need to stay alive," he explains.

Lindegren and researchers at Biosys, a biotechnology company in California, have shown that nematodes can be dried out—harmlessly—by osmotic desiccation. "They're still alive but dormant," he says, and they can be revived any time up to 30 days later by simply adding water."

Osmotic desiccation may be the quickest and most convenient way to handle the large numbers of nematodes required for pest control. For example, experiments by Lindegren and others show that 225 to 500 million nematodes per acre are needed to reduce navel orangeworm populations in almond orchards.

Nematodes are dried by dunking them in an osmolytic solution, that is, a solution in which there is less water than inside the nematodes. Because water seeks an equilibrium, water molecules in the worms will move out of their bodies into the solution.

Once the nematodes are dried to just the right point, the solution is filtered off, and the resulting "paste" of now very flat worms can be refrigerated in containers at about 45°F. When water is added, the flattened worms plump back up again. When they start moving around, the nematodes are ready to be sprayed onto plant, tree, or soil.

"In looking back on our research, we realized that we'd inadvertently duplicated what we think happens in the nature," says Lindegren. Nematodes, when not inside an insect host, live in the thin film of water that surrounds soil particles. "When the soil starts to dry out, the concentration of osmotic compounds such as salts in the film increases, pulling water out of the worms and into the film. When moisture gets back into the soil, through irrigation or rain, the process is reversed—the high concentration of osmotic compounds inside the nematode's body pulls in water from the soil and the worm revives."

Beneficial nematodes push into the larval or wormlike stage of pests through openings such as mouth, breathing tubes, or anus. Once inside, the nematodes excrete a unique bacteria that kills the host, serves as food for the nematodes, and produces antibiotics that keep other bacteria at bay. Inside this snug and comfortable shelter, the nematodes feed and quickly develop into adults that mate and lay eggs. In subsequent generations, overcrowding occurs as more young nematodes hatch from these eggs. Their shelter literally bursts at its seams ejecting the lively offspring into the soil.

For technical information, contact James E. Lindegren, USDA-ARS Horticultural Crops Research Laboratory, 2021 South Peach Avenue, Fresno, CA 93727 (209) 453-3000. *Patent Application Serial No. 07/034,883, "Storage and Shipment of Osmotically Desiccated Entomogenous Nematodes."*

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